NTP Research Concept: Trimethylsilyldiazomethane

Project Leader

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Background and Rationale

Trimethylsilyldiazomethane (TMSD) is a synthetic methylating reagent used for organic synthesis and in analytical methods (e.g., gas chromatography). TMSD [$(CH_3)_3SiCHN_2$] is commonly used for the derivatization of carboxylic acid groups (ROOH) into methyl esters ($ROOCH_3$). TMSD was originally developed as a less toxic substitute for the related compound diazomethane ($CH2N_2$) (1), which is highly flammable (gas/air mixtures are explosive) and is known to cause severe pulmonary injury and death after inhalation exposure in humans (2). An acute inhalation toxicity study has been described for diazomethane (2) in which rabbits were exposed to atmospheres containing 2-12 ppm diazomethane (for 5-20 min, 1-4 times). This exposure regimen induced bronchopneumonia and death prior to day 7. It was also reported that inhalation exposure of cats to diazomethane at 175 ppm for 10 min was lethal within 3 days (2). Furthermore, diazomethane has been described as carcinogenic and tumorigenic to the lung (2, 3). The American Conference of Industrial Hygienists (ACGIH) has classified diazomethane as a suspected human carcinogen (2); however, the International Agency for Research on Cancer (IARC) has it listed as not classifiable (2).

There are very little data regarding the toxicity of TMSD. TMSD has been reported to be a skin irritant, to be harmful if ingested, to target multiple organs and to be a suspected reproductive toxicant (4-6). These effects of TMSD have been reported in Material Safety Data Sheets (MSDSs) provided by chemical suppliers; however, supporting data are not available. Exposure to TMSD in an occupational setting (e.g. chemical laboratory) is likely to occur via dermal contact or inhalation. TMSD is marketed as a safe alternative to diazomethane; however, this may not be entirely true. No inhalation studies with TMSD have been performed to date.

The Occupational Safety and Health Administration (OSHA) nominated TMSD largely as a result of the deaths in 2008 and 2009 of two chemists who were exposed to TMSD in the workplace (7, 8), as well as the known inhalation toxicity of the related compound diazomethane and the lack of toxicity data for TMSD. Both deaths were a result of progressive respiratory distress from pulmonary injury and edema believed to be caused by inhalation exposure to TMSD. It is unknown whether the pulmonary effects were caused by TMSD, diazomethane generated by the breakdown of TMSD, or a metabolite of TMSD. Therefore, there is a need to characterize the inhalation toxicity of TMSD in order to update MSDSs and other chemical reviews and to establish appropriate inhalation exposure limits. The occupational exposure limit (ACGIH TLV, OSHA PEL and NIOSH REL) for diazomethane is (TWA) 0.2 ppm. This concentration

was chosen based on limited exposure data and because diazomethane has a similar mechanism of toxicity to that of phosgene (2).

Key Issues

1. Health and safety of individuals performing stability and toxicity studies with TMSD. Conservative safety precautions will be taken to protect those individuals involved in this study.
2. The stability and breakdown of TMSD into diazomethane and/or other chemical species.
3. The effect of the solvent carrier for TMSD on toxicity and in the generation of vapor.
4. The feasibility of generating a specific atmospheric concentration of pure TMSD vapor in order to perform an acute inhalation toxicity study.
5. Nose-only versus whole body exposure and breathing differences between rodents and humans. Nose-only exposure will be used if the study is done within the NTP/NIEHS in-house inhalation facility due to containment and safety concerns.

Specific Aims

1. Characterize the availability, purity (solvents) and stability of TMSD.
2. Determine the feasibility of generating controlled atmospheric concentrations of pure (and stable) TMSD vapor and characterize the stability of TMSD in artificial lung fluid.
3. Perform acute inhalation toxicity study.
4. Perform additional *in vitro* or *in vivo* studies with TMSD.

Proposed Approach

Because there is limited information available for TMSD, the issues described below (1 and 2) need to be addressed prior to performing an inhalation study (3).

Specific Aim 1. Screen for bulk liquid purity and stability of TMSD from different vendors. TMSD has been reported to be stable as a neat solution, or in a hydrocarbon vehicle (9). Pure TMSD may not be commercially available since most applications use TMSD in hexane or diethyl ether solvent. A preliminary search of suppliers has shown that TMSD is mainly available in liquid form: in methylene chloride, diethyl ether or hexane. The generation of TMSD vapors in the presence of a solvent carrier could be problematic. In addition, the effects of the hydrocarbon carrier/vehicle on toxicity must be considered.

Specific Aim 2. Investigate the feasibility of generating a specified atmospheric concentration of TMSD vapor in an inhalation chamber without animals and assess the stability of TMSD vapor within the chamber. It is possible that TMSD may break down into diazomethane and/or other chemical species. The stability of TMSD in artificial

lung fluid will also be assessed to determine whether TMSD may break down into diazomethane and/or other chemical species upon contact with liquid lining the lung surface.

Specific Aim 3. If we find that TMSD is stable enough to consistently generate TMSD vapor within the inhalation chamber, then a 14-day inhalation study will be performed using nose-only or whole body exposure (5 days per week, 6 hr per day) to assess the acute lung toxicity of TMSD in rats and mice (male and female). This range-finding study would include 0.2 ppm, the occupational exposure limit for diazomethane. Because the respiratory tract appears to be the likely target site of acute exposure, the lungs will be collected from all animals upon completion of the exposure regimen for histopathologic evaluation.

Specific Aim 4. Additional *in vitro* or acute *in vivo* studies may be conducted with TMSD in order to evaluate inhalation toxicokinetics, dermal toxicity (skin irritation and corrosion), eye irritation and the mechanism of TMSD-induced pulmonary toxicity. Because of its acute toxicity, a subchronic inhalation study with TMSD does not appear to be warranted at this time. However, depending on the results of the acute toxicity study, we may consider performing a subchronic inhalation toxicity study with TMSD to evaluate the effects of routine, low dose, longer term exposure to chemists in a laboratory setting.

Significance and Expected Outcome

If TMSD vapor is found to be stable, then an acute inhalation toxicity study may be feasible. Data obtained from these studies could be used to update the MSDS (as well as other chemical reviews) for TMSD and to establish an inhalation occupational exposure limit. Ultimately these toxicity data could be used to inform risk assessment and regulatory actions for TMSD. If TMSD is found to be unstable and to readily form diazomethane, then an acute inhalation toxicity study will likely not be necessary. Diazomethane has previously been shown to be highly toxic (and lethal) upon inhalation. An occupational exposure limit for diazomethane is already established and can be used to provide further risk assessment and regulation of TMSD. Further evaluation will be needed if TMSD breaks down to yield toxic products other than diazomethane.

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